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<120> DNA POLYMERASES

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<141> 2003-04-15

<160> 33

<170> PatentIn version 3.1

<210> 1

<211> 776

<212> PRT

<213> Unknown

<220>

<223> Variant derived from Pyrococcus furiosus Pfu-Polymerase

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Arg Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Arg Asp Asp Ser Lys 35 40 45

Ile Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val 50 60

Arg Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro 65 7.0 75 80

Ile Thr Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Thr 85 90 95

Ile Arg Glu Lys Val Arg Glu His Pro Ala Val Val Asp Ile Phe Glu 100 105 110

Tyr Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile 115 120 125

Pro Met Glu Glu Glu Glu Leu Lys Ile Leu Ala Phe Asp Ile Glu 130 135 140

Thr Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met 145 150 155 160

Ile Ser Tyr Ala Asp Glu Asn Glu Ala Lys Val Ile Thr Trp Lys Asn 165 170 175

Ile Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile 180 185 190

Lys Arg Phe Leu Arg Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Val 195 200 205

Thr Tyr Asn Gly Asp Ser Phe Asp Phe Pro Tyr Leu Ala Lys Arg Ala 210 215 220

Glu Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro 235 235 240

Lys Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg 245 250 255

Ile His Phe Asp Leu Tyr His Val Ile Thr Arg Thr Ile Asn Leu Pro 260 265 270

Thr Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys 275 280 285

Glu Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Ser Gly Glu 290 295 300 Page 2

Asn Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Arg 325 330 335 Leu Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Val 355 360 365 Ala Pro Asn Lys Pro Ser Glu Glu Glu Tyr Gln Arg Arg Leu Arg Glu 370 375 380 Ser Tyr Thr Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu 385 390 395 400 Asn Ile Val Tyr Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr His Asn Val Ser Pro Asp Thr Leu Asn Leu Glu Gly Cys Lys Asn 420 425 430 Tyr Asp Ile Ala Pro Gln Val Gly His Lys Phe Cys Lys Asp Ile Pro 435 440 445 Gly Phe Ile Pro Ser Leu Leu Gly His Leu Leu Glu Glu Arg Gln Lys 450 460 Ile Lys Thr Lys Met Lys Glu Thr Gln Asp Pro Ile Glu Lys Ile Leu 465 470 475 480 Leu Asp Tyr Arg Gln Lys Ala Ile Lys Leu Leu Ala Asn Ser Phe Tyr 495 Gly Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala 500 510 Glu Ser Val Thr Ala Trp Gly Arg Lys Tyr Ile Glu Leu Val Trp Lys 515 520 525 515 Glu Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp 530 540 Gly Leu Tyr Ala Thr Ile Pro Gly Gly Glu Ser Glu Glu Ile Lys Lys Page 3

Lys Ala Leu Glu Phe Val Lys Tyr Ile Asn Ser Lys Leu Pro Gly Leu 565 570 575

Leu Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr 580 585 590

Lys Lys Arg Tyr Ala Val Ile Asp Glu Glu Gly Lys Val Ile Thr Arg 595 600 605

Gly Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr 610 615 620

Gln Ala Arg Val Leu Glu Thr Ile Leu Lys His Gly Asp Val Glu Glu 625 630 635 640

Ala Val Arg Ile Val Lys Glu Val Ile Gln Lys Leu Ala Asn Tyr Glu 645 650 655

Ile Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu 660 665 670

His Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Lys Leu 675 680 685

Ala Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile 690 695 700

Val Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu 705 710 715 720

Glu Tyr Asp Pro Lys Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu 725 730 735

Asn Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr 740 745 750

Arg Lys Glu Asp Leu Arg Tyr Gln Lys Thr Arg Gln Val Gly Leu Thr 755 760 765

Ser Trp Leu Asn Ile Lys Lys Ser 770 775

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Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Arg Asp Asp Ser Lys Ile 35 40 45

Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val Arg 50 55 60

Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro Ile 70 75 80

Thr Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Thr Ile 85 90 95

Arg Glu Lys Val Arg Glu His Pro Ala Val Val Asp Ile Phe Glu Tyr 100 105 110

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro 115 120 125

Met Glu Gly Glu Glu Leu Lys Ile Leu Ala Phe Asp Ile Glu Thr 130 135 140

Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile 145 150 155 160

Ser Tyr Ala Asp Glu Asn Glu Ala Lys Val Ile Thr Trp Lys Asn Ile 165 170 175

Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys 180 185 190

Arg Phe Leu Arg Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Val Thr 195 200 205

Tyr Asn Gly Asp Ser Phe Asp Phe Pro Tyr Leu Ala Lys Arg Ala Glu 210 215 220

Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys 235 230 240 Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile 245 250 255 His Phe Asp Leu Tyr His Val Ile Thr Arg Thr Ile Asn Leu Pro Thr 260 265 270 Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu 275 280 285 Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Ser Gly Glu Asn 290 295 300 Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr 305 310 315 320 Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Arg Leu 325 330 335 Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu 340 345 350 Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Val Ala 355 360 365 Pro Asn Lys Pro Ser Glu Glu Glu Tyr Gln Arg Arg Leu Arg Glu Ser 370 380 Tyr Thr Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn 385 390 395 400 Ile Val Tyr Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr 405 410 415 His Asn Val Ser Pro Asp Thr Leu Asn Leu Glu Gly Cys Lys Asn Tyr 420 425 430 Asp Ile Ala Pro Gln Val Gly His Lys Phe Cys Lys Asp Ile Pro Gly 435 440 445 Phe Ile Pro Ser Leu Leu Gly His Leu Leu Glu Glu Arg Gln Lys Ile 450 455 460 Lys Thr Lys Met Lys Glu Thr Gln Asp Pro Ile Glu Lys Ile Leu Leu 465 470 475 480 Page 6

Asp Tyr Arg Gln Lys Ala Ile Lys Leu Leu Ala Asn Ser Phe Tyr Gly
485 490 495 Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu 500 510 Ser Val Thr Ala Trp Gly Arg Lys Tyr Ile Glu Leu Val Trp Lys Glu 515 520 525 Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly 530 540 Leu Tyr Ala Thr Ile Pro Gly Gly Glu Ser Glu Glu Ile Lys Lys 545 550 555 560 Ala Leu Glu Phe Val Lys Tyr Ile Asn Ser Lys Leu Pro Gly Leu Leu 565 570 575 Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys 580 585 590 Lys Arg Tyr Ala Val Ile Asp Glu Glu Gly Lys Val Ile Thr Arg Gly 595 600 605 Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln 610 615 620 Ala Arg Val Leu Glu Thr Ile Leu Lys His Gly Asp Val Glu Glu Ala 625 630 635 640 Val Arg Ile Val Lys Glu Val Ile Gln Lys Leu Ala Asn Tyr Glu Ile 645 650 655 Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His 660 665 670 Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Lys Leu Ala 675 680 685 Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val 690 695 700 Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu 705 710 715 720 Tyr Asp Pro Lys Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Page 7

735

Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg 740 745 750

Lys Glu Asp Leu Arg Tyr Gln Lys Thr Arg Gln Val Gly Leu Thr Ser 755 760 765

Trp Leu Asn Ile Lys Lys Ser 770 775

<210> 3

<211> 776

<212> PRT

<213> Unknown

<220>

<223> Variant derived from Pyrococcus furiosus Pfu-Polymerase

<400> 3

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5 10 15

Ile Arg Leu Phe Lys Lys Glu Asn Gly Lys Phe Lys Ile Glu His Asp 20 25 30

Arg Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Arg Asp Asp Ser Lys 35 40 45

Ile Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val 50 60

Arg Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro 65 70 75 80

Ile Thr Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Thr 85 90 95

Ile Arg Glu Lys Val Arg Glu His Pro Ala Val Val Asp Ile Phe Glu 100 105 110

Tyr Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile 115 120 125

20510452_1(8.21.08).TXT Pro Met Glu Gly Glu Glu Leu Lys Ile Leu Ala Phe Asp Ile Glu Thr Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met 145 150 155 160 Ile Ser Tyr Ala Asp Glu Asn Glu Ala Lys Val Ile Thr Trp Lys Asn 165 170 175 Ile Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys Arg Phe Leu Arg Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Val 195 200 205 Thr Tyr Asn Gly Asp Ser Phe Asp Phe Pro Tyr Leu Ala Lys Arg Ala 210 215 220 Glu Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro 225 230 235 240 Lys Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg 245 250 255 Ile His Phe Asp Leu Tyr His Val Ile Thr Arg Thr Ile Asn Leu Pro 260 265 270 Thr Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys 275 280 285 Glu Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Ser Gly Glu 290 295 300 Asn Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr 305 310 315 320 Tyr Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Arg 325 330 335 Leu Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn 340 345 350 Leu Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Val 355 360 365 Ala Pro Asn Lys Pro Ser Glu Glu Glu Tyr Gln Arg Arg Leu Arg Glu 370 375 380

Ser Tyr Thr Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu 385 390 395 400 Asn Ile Val Tyr Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr His Asn Val Ser Pro Asp Thr Leu Asn Leu Glu Gly Cys Lys Asn 420 425 430 Tyr Asp Ile Ala Pro Gln Val Gly His Lys Phe Cys Lys Asp Ile Pro 435 440 445 Gly Phe Ile Pro Ser Leu Leu Gly His Leu Leu Glu Glu Arg Gln Lys Ile Lys Thr Lys Met Lys Glu Thr Gln Asp Pro Ile Glu Lys Ile Leu 465 470 475 480 Leu Asp Tyr Arg Gln Lys Ala Ile Lys Leu Leu Ala Asn Ser Phe Tyr Gly Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala 500 505 510 Glu Ser Val Thr Ala Trp Gly Arg Lys Tyr Ile Glu Leu Val Trp Lys 515 520 525 Glu Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp 530 540 Gly Leu Tyr Ala Thr Ile Pro Gly Gly Glu Ser Glu Glu Ile Lys Lys 545 550 555 560 Lys Ala Leu Glu Phe Val Lys Tyr Ile Asn Ser Lys Leu Pro Gly Leu
565
570 Leu Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr 580 585 590 Lys Lys Arg Tyr Ala Val Ile Asp Glu Glu Gly Lys Val Ile Thr Arg 595 600 605 Gly Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr 610 620 Gln Ala Arg Val Leu Glu Thr Ile Leu Lys His Gly Asp Val Glu Glu 635 Page 10

Ala Val Arg Ile Val Lys Glu Val Ile Gln Lys Leu Ala Asn Tyr Glu 645 650 655

Ile Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu 660 665 670

His Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Lys Leu 675 680 685

Ala Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile 690 695 700

Val Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu 705 710 715 720

Glu Tyr Asp Pro Lys Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu 725 730 735

Asn Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr 740 745 750

Arg Lys Glu Asp Leu Arg Tyr Gln Lys Thr Arg Gln Val Gly Leu Thr 755 760 765

Ser Trp Leu Asn Ile Lys Lys Ser 770 775

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<211> 776

<212> PRT

<213> Unknown

<220>

<223> Variant derived from Pyrococcus furiosus Pfu-Polymerase

<400> 4

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Ile Arg Leu Phe Lys Lys Glu Asn Gly Lys Phe Lys Ile Glu His Asp 20 25 30

Arg Thr Phe Arg Pro Ala Ile Tyr Ala Leu Leu Arg Asp Asp Ser Lys Page 11 Ile Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val 50 60 Arg Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro 65 70 75 80 Ile Thr Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Thr 85 90 95 Ile Arg Glu Lys Val Arg Glu His Pro Ala Val Val Asp Ile Phe Glu 100 105 110 Tyr Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile 115 120 125 Pro Met Glu Glu Glu Glu Leu Lys Ile Leu Ala Phe Asp Ile Glu 130 135 140 Thr Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met 145 150 155 160 Ile Ser Tyr Ala Asp Glu Asn Glu Ala Lys Val Ile Thr Trp Lys Asn 165 170 175 Ile Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys Arg Phe Leu Arg Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Val 195 200 205 Thr Tyr Asn Gly Asp Ser Phe Asp Phe Pro Tyr Leu Ala Lys Arg Ala 210 215 220 Glu Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro 225 230 235 240 Lys Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg 245 250 255 Ile His Phe Asp Leu Tyr His Val Ile Thr Arg Thr Ile Asn Leu Pro 260 265 270

Thr Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys 275 280 285

20510452_1(8.21.08).TXT Glu Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Ser Gly Glu Asn Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr 305 310 315 Tyr Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Arg 325 330 335 Leu Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn 340 350 Leu Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Val Ala Pro Asn Lys Pro Ser Glu Glu Glu Tyr Gln Arg Arg Leu Arg Glu 370 380 Ser Tyr Thr Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn Ile Val Tyr Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile 410 Thr His Asn Val Ser Pro Asp Thr Leu Asn Leu Glu Gly Cys Lys Asn 420 425 430 Tyr Asp Ile Ala Pro Gln Val Gly His Lys Phe Cys Lys Asp Ile Pro 435 440 445 Gly Phe Ile Pro Ser Leu Leu Gly His Leu Leu Glu Glu Arg Gln Lys Ile Lys Thr Lys Met Lys Glu Thr Gln Asp Pro Ile Glu Lys Ile Leu 465 470 475 480 Leu Asp Tyr Arg Gln Lys Ala Ile Lys Leu Leu Ala Asn Ser Phe Tyr 490 495 Gly Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala 500 510 Glu Ser Val Thr Ala Trp Gly Arg Lys Tyr Ile Glu Leu Val Trp Lys 515 520 525 Glu Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp 530 540

Gly Leu Tyr Ala Thr Ile Pro Gly Gly Glu Ser Glu Glu Ile Lys Lys 545 550 555

Lys Ala Leu Glu Phe Val Lys Tyr Ile Asn Ser Lys Leu Pro Gly Leu 565 570 575

Leu Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr 580 585 590

Lys Lys Arg Tyr Ala Val Ile Asp Glu Glu Gly Lys Val Ile Thr Arg 595 600 605

Gly Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr 610 615 620

Gln Ala Arg Val Leu Glu Thr Ile Leu Lys His Gly Asp Val Glu Glu 625 630 635 640

Ala Val Arg Ile Val Lys Glu Val Ile Gln Lys Leu Ala Asn Tyr Glu 645 650 655

Ile Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu 660 665 670

His Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Lys Leu 675 680 685

Ala Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile 690 695 700

Val Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu 705 710 715 720

Glu Tyr Asp Pro Lys Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu 725 730 735

Asn Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr 740 745 750

Arg Lys Glu Asp Leu Arg Tyr Gln Lys Thr Arg Gln Val Gly Leu Thr
755 760 765

Ser Trp Leu Asn Ile Lys Lys Ser 770 775

<210> 5

<211> 776

<212> PRT

<213> Unknown

<220>

<223> Variant derived from Pyrococcus furiosus Pfu-Polymerase <400> 5

Met Ala Ile Leu Asp Val Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val 1 5 10 15

Ile Arg Leu Phe Lys Lys Glu Asn Gly Lys Phe Lys Ile Glu His Asp 20 25 30

Arg Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Arg Asp Asp Ser Lys 35 40 45

Ile Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val 50 60

Arg Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro 65 70 75 80

Ile Thr Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Gln Pro Thr 85 90 95

Ile Arg Glu Lys Val Arg Glu His Pro Ala Val Val Asp.Ile Phe Glu 100 105 110

Tyr Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile 115 120 125

Pro Met Glu Glu Glu Glu Leu Lys Ile Leu Ala Phe Asp Ile Glu 130 135 140

Thr Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met 145 150 155 160

Ile Ser Tyr Ala Asp Glu Asn Glu Ala Lys Val Ile Thr Trp Lys Asn 165 170 175

Ile Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile 180 185 190

Lys Arg Phe Leu Arg Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Val Page 15

Tyr Asn Gly Asp Ser Phe Asp Phe Pro Tyr Leu Ala Lys Arg Ala 210 220 Glu Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro 225 230 235 240 Lys Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg 245 250 255 Ile His Phe Asp Leu Tyr His Val Ile Thr Arg Thr Ile Asn Leu Pro 260 265 270 Thr Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys 275 280 285 Glu Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Ser Gly Glu 290 295 300 Asn Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr 305 310 315 320 Tyr Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Arg 325 330 335 Leu Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn 340 345 350Leu Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Val 355 360 365 Ala Pro Asn Lys Pro Ser Glu Glu Glu Tyr Gln Arg Arg Leu Arg Glu 370 380 Ser Tyr Thr Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu 385 390 395 400 Asn Ile Val Tyr Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr His Asn Val Ser Pro Asp Thr Leu Asn Leu Glu Gly Cys Lys Asn 420 425 430 Tyr Asp Ile Ala Pro Gln Val Gly His Lys Phe Cys Lys Asp Ile Pro 435 440 445 Gly Phe Ile Pro Ser Leu Leu Gly His Leu Leu Glu Glu Arg Gln Lys 455 Ile Lys Thr Lys Met Lys Glu Thr Gln Asp Pro Ile Glu Lys Ile Leu Leu Asp Tyr Arg Gln Lys Ala Ile Lys Leu Leu Ala Asn Ser Phe Tyr Gly Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala 500 510 Glu Ser Val Thr Ala Trp Gly Arg Lys Tyr Ile Glu Leu Val Trp Lys 515 520 525 Glu Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp 530 540 Gly Leu Tyr Ala Thr Ile Pro Gly Gly Glu Ser Glu Glu Ile Lys Lys 545 550 560 Lys Ala Leu Glu Phe Val Lys Tyr Ile Asn Ser Lys Leu Pro Gly Leu 565 570 575 Leu Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr
580 585 590 Lys Lys Arg Tyr Ala Val Ile Asp Glu Glu Gly Lys Val Ile Thr Arg 595 600 605 Gly Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr 610 615 620 Gln Ala Arg Val Leu Glu Thr Ile Leu Lys His Gly Asp Val Glu Glu Ala Val Arg Ile Val Lys Glu Val Ile Gln Lys Leu Ala Asn Tyr Glu 645 650 655 Ile Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Lys Leu Ala Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile

Val Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu 705 710 715 720

Glu Tyr Asp Pro Lys Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu 725 730 735

Asn Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr 740 745 750

Arg Lys Glu Asp Leu Arg Tyr Gln Lys Thr Arg Gln Val Gly Leu Thr 755 760 765

Ser Trp Leu Asn Ile Lys Lys Ser 770 775

<210> 6

<211> 776

<212> PRT

<213> Unknown

<220>

<223> Variant derived from Pyrococcus furiosus Pfu-Polymerase <400> 6

Met Ala Ile Leu Asp Val Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val
1 5 10 15

Ile Arg Leu Phe Lys Lys Glu Asn Gly Lys Phe Lys Ile Glu His Asp $20 \hspace{1cm} 25 \hspace{1cm} 30$.

Arg Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Arg Asp Asp Ser Lys 35 40 45

Ile Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val 50 60

Arg Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro 65 70 75 80

Ile Thr Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Arg Pro Thr 85 90 95

Ile Arg Glu Lys Val Arg Glu His Pro Ala Val Val Asp Ile Phe Glu 100 105 110 Page 18

Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile 115 120 125 Pro Met Glu Gly Glu Glu Leu Lys Ile Leu Ala Phe Asp Ile Glu 130 Thr Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met 145 150 155 160 Ile Ser Tyr Ala Asp Glu Asn Glu Ala Lys Val Ile Thr Trp Lys Asn 165 170 175 Ile Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys Arg Phe Leu Arg Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Val 195 200 205 Thr Tyr Asn Gly Asp Ser Phe Asp Phe Pro Tyr Leu Ala Lys Arg Ala 210 215 220 Glu Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro 235 230 240 Lys Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg 245 250 255 Ile His Phe Asp Leu Tyr His Val Ile Thr Arg Thr Ile Asn Leu Pro $260 \hspace{1cm} 265 \hspace{1cm} 270$ Thr Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys 275 280 285 Glu Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Ser Gly Glu 290 295 300 Asn Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr 305 310 315 320 Tyr Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Arg 325 330 335 Leu Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn 340 345 350 Leu Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Val Page 19

Ala Pro Asn Lys Pro Ser Glu Glu Glu Tyr Gln Arg Arg Leu Arg Glu 370 380 ser Tyr Thr Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn Ile Val Tyr Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr His Asn Val Ser Pro Asp Thr Leu Asn Leu Glu Gly Cys Lys Asn 420 425 430 Tyr Asp Ile Ala Pro Gln Val Gly His Lys Phe Cys Lys Asp Ile Pro 435 440 445 Gly Phe Ile Pro Ser Leu Leu Gly His Leu Leu Glu Glu Arg Gln Lys Ile Lys Thr Lys Met Lys Glu Thr Gln Asp Pro Ile Glu Lys Ile Leu Leu Asp Tyr Arg Gln Lys Ala Ile Lys Leu Leu Ala Asn Ser Phe Tyr Gly Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala 500 510 Glu Ser Val Thr Ala Trp Gly Arg Lys Tyr Ile Glu Leu Val Trp Lys 515 520 525 Glu Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp 530 540 Gly Leu Tyr Ala Thr Ile Pro Gly Gly Glu Ser Glu Glu Ile Lys Lys 545 550 555 560 Lys Ala Leu Glu Phe Val Lys Tyr Ile Asn Ser Lys Leu Pro Gly Leu 565 570 575 Leu Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr 580 585 590 Lys Lys Arg Tyr Ala Val Ile Asp Glu Glu Gly Lys Val Ile Thr Arg 595 600 605 20510452_1(8.21.08).TXT
Gly Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr
610 615 620

Gln Ala Arg Val Leu Glu Thr Ile Leu Lys His Gly Asp Val Glu Glu 625 630 635 640

Ala Val Arg Ile Val Lys Glu Val Ile Gln Lys Leu Ala Asn Tyr Glu 645 650 655

Ile Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu 660 665 670

His Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Lys Leu 675 680 685

Ala Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile 690 695 700

Val Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu 705 710 715 720

Glu Tyr Asp Pro Lys Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu 725 730 735

Asn Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr . 740 745 750

Arg Lys Glu Asp Leu Arg Tyr Gln Lys Thr Arg Gln Val Glý Leu Thr 755 760 765

Ser Trp Leu Asn Ile Lys Lys Ser 770 775

<210> 7

<211> 776

<212> PRT

<213> Unknown

<220>

<223> Variant derived from Pyrococcus furiosus Pfu-Polymerase <400> 7

Met Ala Ile Leu Asp Val Asp Ala Ile Thr Glu Glu Gly Lys Pro Val 1 5 10 15

Ile Arg Leu Phe Lys Lys Glu Asn Gly Lys Phe Lys Ile Glu His Asp 20 25 30 Arg Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Arg Asp Asp Ser Lys 35 40 45 Ile Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val 50 55 60 Arg Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro 65 70 75 80 Ile Thr Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Thr 85 . 90 95 Ile Arg Glu Lys Val Arg Glu His Pro Ala Val Val Asp Ile Phe Glu
100 105 110 Tyr Asp Arg Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile 115 120 125 Pro Met Glu Glu Glu Glu Leu Lys Ile Leu Ala Phe Asp Ile Glu 130 Thr Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met 145 150 155 160 160 Ile Ser Tyr Ala Asp Glu Asn Glu Ala Lys Val Ile Thr Trp Lys Asn 165 170 175 Ile Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile 180 185 190Lys Arg Phe Leu Arg Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Val 195 200 205 Thr Tyr Asn Gly Asp Ser Phe Asp Phe Pro Tyr Leu Ala Lys Arg Ala 210 215 220 Glu Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro 225 230 235 240 Lys Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg 245 250 255 Ile His Phe Asp Leu Tyr His Val Ile Thr Arg Thr Ile Asn Leu Pro 260 265 270 Page 22

Thr Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys 275 280 285 Glu Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Ser Gly Glu 290 295 300 Asn Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr 305 310 315 320 Tyr Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Arg 325 330 335Leu Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn 340 345 350 Leu Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Val 355 360 365 Ala Pro Asn Lys Pro Ser Glu Glu Glu Tyr Gln Arg Arg Leu Arg Glu 370 375 380 Ser Tyr Thr Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu 385 390 395 400 Asn Ile Val Tyr Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr His Asn Val Ser Pro Asp Thr Leu Asn Leu Glu Gly Cys Lys Asn 420 425 430 Tyr Asp Ile Ala Pro Gln Val Gly His Lys Phe Cys Lys Asp Ile Pro 435 440 445 Gly Phe Ile Pro Ser Leu Leu Gly His Leu Leu Glu Glu Arg Gln Lys 450 455 460 Ile Lys Thr Lys Met Lys Glu Thr Gln Asp Pro Ile Glu Lys Ile Leu 465 470 475 480 465 Leu Asp Tyr Arg Gln Lys Ala Ile Lys Leu Leu Ala Asn Ser Phe Tyr Gly Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala 500 505 510 Glu Ser Val Thr Ala Trp Gly Arg Lys Tyr Ile Glu Leu Val Trp Lys Page 23

Glu Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp 530 540 Gly Leu Tyr Ala Thr Ile Pro Gly Gly Glu Ser Glu Glu Ile Lys Lys 545 550 555 560 Lys Ala Leu Glu Phe Val Lys Tyr Ile Asn Ser Lys Leu Pro Gly Leu 565 570 575 Leu Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr 580 585 590 Lys Lys Arg Tyr Ala Val Ile Asp Glu Glu Gly Lys Val Ile Thr Arg 595 600 605 Gly Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr 610 615 620 Gln Ala Arg Val Leu Glu Thr Ile Leu Lys His Gly Asp Val Glu Glu Ala Val Arg Ile Val Lys Glu Val Ile Gln Lys Leu Ala Asn Tyr Glu 645 650 655 Ile Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu 660 665 670 His Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Lys Leu 675 680 685 Ala Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu 705 710 715 720 Glu Tyr Asp Pro Lys Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu 725 730 735 Asn Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr 740 745 750 Lys Glu Asp Leu Arg Tyr Gln Lys Thr Arg Gln Val Gly Leu Thr 755 760 765

Ser Trp Leu Asn Ile Lys Lys Ser 770 775

<210> 8

<211> 776

<212> PRT

<213> Unknown

<220>

<223> Variant derived from Pyrococcus furiosus Pfu-Polymerase <400> 8

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Ile Arg Leu Phe Lys Lys Glu Asn Gly Lys Phe Lys Ile Glu His Asp 20 25 30

Arg Thr Phe Arg Pro Ala Ile Tyr Ala Leu Leu Arg Asp Asp Ser Lys 35 40 45

Ile Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val 50 55 60

Arg Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro 65 70 75 80

Ile Thr Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Thr 85 90 95

Ile Arg Glu Lys Val Arg Glu His Pro Ala Val Val Asp Ile Phe Glu 100 105 110

Tyr Asp Gln Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile 115 120 125

Pro Met Glu Gly Glu Glu Leu Lys Ile Leu Ala Phe Asp Ile Glu 130 135 140

Thr Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met 145 150 155 160

Ile Ser Tyr Ala Asp Glu Asn Glu Ala Lys Val Ile Thr Trp Lys Asn 165 170 175

Ile Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys Arg Phe Leu Arg Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Val 195 200 205 Thr Tyr Asn Gly Asp Ser Phe Asp Phe Pro Tyr Leu Ala Lys Arg Ala 210 215 220 Glu Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro 225 230 235 240 Lys Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg 245 250 255 Ile His Phe Asp Leu Tyr His Val Ile Thr Arg Thr Ile Asn Leu Pro 260 265 270 Thr Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys 275 280 285 Glu Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Ser Gly Glu 290 295 300 Asn Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr 305 310 315 320 Tyr Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Arg 325 330 335 Leu Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn 340 345 350 Leu Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Val 355 360 365 Ala Pro Asn Lys Pro Ser Glu Glu Glu Tyr Gln Arg Arg Leu Arg Glu 370 380 Ser Tyr Thr Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu 385 390 395 400 Asn Ile Val Tyr Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile 405 410 415 Thr His Asn Val Ser Pro Asp Thr Leu Asn Leu Glu Gly Cys Lys Asn 420 425 430 Page 26

Tyr Asp Ile Ala Pro Gln Val Gly His Lys Phe Cys Lys Asp Ile Pro Gly Phe Ile Pro Ser Leu Leu Gly His Leu Leu Glu Glu Arg Gln Lys Ile Lys Thr Lys Met Lys Glu Thr Gln Asp Pro Ile Glu Lys Ile Leu 465 470 475 480 Leu Asp Tyr Arg Gln Lys Ala Ile Lys Leu Leu Ala Asn Ser Phe Tyr Gly Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala 500 510 Glu Ser Val Thr Ala Trp Gly Arg Lys Tyr Ile Glu Leu Val Trp Lys 515 520 525 Glu Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp 530 540 Gly Leu Tyr Ala Thr Ile Pro Gly Gly Glu Ser Glu Glu Ile Lys Lys 545 550 555 560 Lys Ala Leu Glu Phe Val Lys Tyr Ile Asn Ser Lys Leu Pro Gly Leu 565 570 575 Leu Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr 580 585 590 Lys Lys Arg Tyr Ala Val Ile Asp Glu Glu Gly Lys Val Ile Thr Arg 595 600 605 Gly Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr 610 620 Gln Ala Arg Val Leu Glu Thr Ile Leu Lys His Gly Asp Val Glu Glu 625 Ala Val Arg Ile Val Lys Glu Val Ile Gln Lys Leu Ala Asn Tyr Glu Ile Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu 660 665 670 His Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Lys Leu Page 27

675

Ala Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile 690 695 700

Val Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu 705 710 715 720

Glu Tyr Asp Pro Lys Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu 725 730 735

Asn Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr 740 745 750

Arg Lys Glu Asp Leu Arg Tyr Gln Lys Thr Arg Gln Val Gly Leu Thr 755 760 765

Ser Trp Leu Asn Ile Lys Lys Ser 770 775

<210> 9

<211> 2328

<212> DNA

<213> Unknown

<220>

Variant derived from Pyrococcus furiosus Pfu-Polymerase <223> <400> 60 atggctatcc tggacgttga cgccatcacc gaagaaggta agccggttat ccgtctgttc aaaaaagaaa acggtaaatt caaaatcgaa cacgaccgta ccttccgtcc gtacatctac 120 gctctgctgc gtgacgactc taaaatcgaa gaagttaaaa aaatcaccgg tgaacgtcat 180 240 ggaaagattg tgagaattgt tgatgtagag aaggttgaga aaaagtttct cggcaagcct 300 attaccgtgt ggaaacttta tttggaacat ccccaagatg ttcccactat tagagaaaaa 360 gttagagaac atccagcagt tgtggacatc ttcgaatacg atattccatt tgcaaagaga 420 tacctcatcg acaaaggcct aataccaatg gagggggaag aagagctaaa gattcttgcc 480 ttcgatatag aaaccctcta tcacgaagga gaagagtttg gaaaaggccc aattataatg attagttatg cagatgaaaa tgaagcaaag gtgattactt ggaaaaacat agatcttcca 540 600 tacgttgagg ttgtatcaag cgagagagag atgataaaga gatttctcag gattatcagg 660 gagaaggatc ctgacattat agttacttat aatggagact cattcgactt cccatattta Page 28

gcgaaaaggg	cagaaaaact	tgggattaaa	ttaaccattg	gaagagatgg	aagcgagccc	720
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<211> 2328

<212> DNA

<213> Unknown

<220>
<223> Variant derived from Pyrococcus furiosus Pfu-Polymerase
<400> 10

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ccagcggtac	ttaggatatt	ggagggattt	ggatacagaa	aggaagacct	cagataccaa	2280
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<210> 11

<211> 2325

<212> DNA

<213> Unknown

<220>

Variant derived from Pyrococcus furiosus Pfu-Polymerase <223> <400> 11 60 atggctatcc tggacgttga ctacatcacc gaagaaggta agccggttat ccgtctgttc 120 aaaaaagaaa acggtaaatt caaaatcgaa cacgaccgta ccttccgtcc gtacatctac 180 gctctgctgc gtgacgactc taaaatcgaa gaagttaaaa aaatcaccgg tgaacgtcat ggaaagattg tgagaattgt tgatgtagag aaggttgaga aaaagtttct cggcaagcct 240 300 attaccgtgt ggaaacttta tttggaacat ccccaagatg ttcccactat tagagaaaaa 360 gttagagaac atccagcagt tgtggacatc ttcgaatacg atatttttgc aaagagatac 420 ctcatcgaca aaggcctaat accaatggag ggggaagaag agctaaagat tcttgccttc 480 gatatagaaa ccctctatca cgaaggagaa gagtttggaa aaggcccaat tataatgatt 540 agttatgcag atgaaaatga agcaaaggtg attacttgga aaaacataga tcttccatac 600 gttgaggttg tatcaagcga gagagagatg ataaagagat ttctcaggat tatcagggag 660 aaggatcctg acattatagt tacttataat ggagactcat tcgacttccc atatttagcg

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<211> 130

<212> PRT

<213> Thermococcus gorgonarius

<400> 12

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Asn Phe Glu Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile 35 40 45

Glu Asp Val Lys Lys Ile Thr Ala Glu Arg His Gly Thr Thr Val Arg 50 55 60

Val Val Arg Ala Glu Lys Val Lys Lys Phe Leu Gly Arg Pro Ile 65 70 75 80

Glu Val Trp Lys Leu Tyr Phe Thr His Pro Gln Asp Val Pro Ala Ile 85 90 95.

Arg Asp Lys Ile Lys Glu His Pro Ala Val Val Asp Ile Tyr Glu Tyr 100 105 110

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro 115 120 125

Met Glu 130

<210> 13

<211> 103

<212> PRT

<213> Unknown

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<400> 13

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Glu Arg Tyr Ile Asp Ser Asn Gly Arg Glu Arg Thr Arg Glu Val Glu 20 25 30

Tyr Lys Pro Ser Leu Phe Ala His Cys Pro Glu Ser Gln Ala Thr Lys 35 40 45

Tyr Phe Asp Ile Tyr Gly Lys Pro Cys Thr Arg Lys Leu Phe Ala Asn 50 60

Met Arg Asp Ala Ser Gln Trp Ile Lys Arg Met Glu Asp Ile Gly Leu 65 70 75 80

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Tyr Asn Tyr Glu Ile Lys Tyr 100

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<211> 44

<212> DNA

<213> Unknown

<220>

<223> Artificial Template

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<210> 16

<211> 22

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24

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<213> Unknown

<220>

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<213> Unknown

<220>

<223> Artificial Template

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44

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<211> 131

<212> PRT

<213> Pyrococcus furiosus

<400> 18

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Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Arg Asp Asp Ser Lys Ile $\frac{35}{40}$

Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val Arg 50 55 60

Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro Ile 65 70 75 80

Thr Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Thr Ile Page 35 Arg Glu Lys Val Arg Glu His Pro Ala Val Val Asp Ile Phe Glu Tyr 100 105 110

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro 115 120 125

Met Glu Gly 130

<210> 19

<211> 131

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<400> 19

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Glu Asp Val Lys Lys Ile Thr Ala Glu Arg His Gly Thr Thr Val Arg 50 60

Val Val Arg Ala Glu Lys Val Lys Lys Lys Phe Leu Gly Arg Pro Ile 65 70 75 80

Glu Val Trp Lys Leu Tyr Phe Thr His Pro Gln Asp Val Pro Ala Ile 85 90 95

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Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro 115 120 125

Met Glu Gly 130 <210> 20

<211> 131

<212> PRT

<213> Pyrococcus kodakaraensis

<400> 20

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Arg Ile Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg 20 25 30

Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile 35 40 45

Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Thr Val Val Thr 50 55 60

Val Lys Arg Val Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Val 65 70 75 80

Glu Val Trp Lys Leu Tyr Phe Thr His Pro Gln Asp Val Pro Ala Ile 85 90 95

Arg Asp Lys Ile Arg Glu His Pro Ala Val Ile Asp Ile Tyr Glu Tyr 100 105 110

Asp Ile Pro Glu Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Val Pro 115 120 125

Met Glu Gly 130

<210> 21

<211> 131

<212> PRT

<213> Desulfurococcus Tok

<400> 21

Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Asp Gly Lys Pro Val Ile 1 5 10 15

Arg Val Phe Lys Lys Glu Lys Gly Glu Phe Lys Ile Asp Tyr Asp Arg 20 25 30

Asp Phe Glu Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile 35 40 45

Glu Asp Ile Lys Lys Ile Thr Ala Glu Arg His Gly Thr Thr Val Arg 50 55 60

Val Thr Arg Ala Glu Arg Val Lys Lys Phe Leu Gly Arg Pro Val 65 70 75 80

Glu Val Trp Lys Leu Tyr Phe Thr His Pro Gln Asp Val Pro Ala Ile 85 90 95

Arg Asp Lys Ile Arg Glu His Pro Ala Val Val Asp Ile Tyr Glu Tyr 100 105 110

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Arg Gly Leu Ile Pro 115 120 125

Met Glu Gly 130

<210> 22

<211> 132

<212> PRT

<213> Thermococcus sp. 9°N-7

<400> 22

Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile 1 5 10 15

Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg 20 25 30

Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile 35 40 45

Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys 50 60

Val Lys Arg Ala Glu Lys Val Gln Lys Lys Glu Phe Leu Gly Arg Pro 65 70 75 80 Page 38

Ile Glu Val Trp Lys Leu Tyr Phe Asn His Pro Gln Asp Val Pro Ala 85 90 95

Ile Arg Asp Arg Ile Arg Ala His Pro Ala Val Val Asp Ile Tyr Glu 100 105 110

Tyr Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile 115 120 125

Pro Met Glu Gly 130

<210> 23

<211> 131

<212> PRT

<213> Thermococcus litoralis

<400> 23

Met Ile Leu Asp Thr Asp Tyr Ile Thr Lys Asp Gly Lys Pro Ile Ile 1 5 10 15

Arg Ile Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Leu Asp Pro 20 25 30

His Phe Gln Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile 35 40 45

Glu Glu Ile Lys Ala Ile Lys Gly Glu Arg His Gly Lys Thr Val Arg 50 55 60

Val Leu Asp Ala Val Lys Val Arg Lys Lys Phe Leu Gly Arg Glu Val 65 70 75 80

Glu Val Trp Lys Leu Ile Phe Glu His Pro Gln Asp Val Pro Ala Met 85 90 95

Arg Gly Lys Ile Arg Glu His Pro Ala Val Val Asp Ile Tyr Glu Tyr 100 105 110

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro 115 120 125

Met Glu Gly

<210> 24

<211> 161

<212> PRT

<213> Methanococcus voltae

<400> 24

Met Asp Leu Asp Tyr Asn Ser Lys Asp Leu Cys Ile Asp Met Tyr Tyr 1 5 10 15

Lys Asn Cys Gly Leu Lys Lys Pro Glu Ile Asn Leu Gln Lys Glu Cys 20 25 30

Glu Phe Lys Pro Tyr Phe Tyr Val Asp Thr Ser Glu Pro Lys Glu Ile 35 40 45

Tyr Asp Tyr Leu Asp Gly Leu Asn Gln Glu Ile Asp Leu Lys Lys Leu 50 60 .

Glu Pro Glu Phe Glu Asn Asn Thr Ser Leu Lys Val Gln Asp Leu Ile 65 . 70 . 75 . 80

Thr Asn Ile Glu Ile Ile Glu Lys Ile Val Tyr Ser Asp Tyr Ile Leu 85 90 95

Asn Gly Lys Asp Ile Ser Glu Val Ser Asp Phe Lys Asn Lys Lys Glu $100 \hspace{1cm} 105 \hspace{1cm} 110$

Arg Lys Ile Cys Lys Val Tyr Val Lys Tyr Pro Asn His Val Lys Ile 115 120 125

Ile Arg Glu Tyr Phe Lys Glu Phe Gly Lys Ser Tyr Glu Phe Asp Ile 130 135 140

Pro Phe Leu Arg Arg Tyr Met Ile Asp Gln Asp Ile Val Pro Ser Ala 145 150 155 160

Lys

<210> 25

<211> 132

<212> PRT

<213> Pyrobaculum islandicum

<400> 25

Met Glu Leu Lys Val Trp Pro Leu Asp Ile Thr Tyr Ala Val Val Gly
1 5 10 15

Ser Val Pro Glu Ile Arg Ile Phe Gly Ile Leu Ser Ser Gly Glu Arg 20 25 30

Val Val Leu Ile Asp Arg Ser Phe Lys Pro Tyr Phe Tyr Val Asp Cys 35 40 45

Ala Val Cys Glu Pro Ala Ala Leu Lys Thr Ala Leu Ser Arg Val Ala 50 55 60

Pro Ile Asp Asp Val Gln Ile Val Glu Arg Arg Phe Leu Gly Arg Ser 70 75 80

Lys Lys Phe Leu Lys Val Ile Ala Lys Ile Pro Glu Asp Val Arg Lys 85 90 95

Leu Arg Glu Ala Ala Met Ser Ile Pro Arg Val Ser Gly Val Tyr Glu $100 \hspace{1cm} 105 \hspace{1cm} 110$

Ala Asp Ile Arg Phe Tyr Met Arg Tyr Met Ile Asp Met Gly Val Val 115 120 125

Pro Cys Ser Trp 130

<210> 26

<211> 131

<212> PRT

<213> Archaeoglobus fulgidus

<400> 26

Met Glu Arg Val Glu Gly Trp Leu Ile Asp Ala Asp Tyr Glu Thr Ile
1 10 15

Gly Gly Lys Ala Val Val Arg Leu Trp Cys Lys Asp Asp Gln Gly Ile 20 25 30

Phe Val Ala Tyr Asp Tyr Asn Phe Asp Pro Tyr Phe Tyr Val Ile Gly 35 40 45

Val Asp Glu Asp Ile Leu Lys Asn Ala Ala Thr Ser Thr Arg Arg Glu 50 55 60

Val Ile Lys Leu Lys Ser Phe Glu Lys Ala Gln Leu Lys Thr Leu Gly 65 70 75 80

Arg Glu Val Glu Gly Tyr Ile Val Tyr Ala His His Pro Gln His Val 85 90 95

Pro Lys Leu Arg Asp Tyr Leu Ser Gln Phe Gly Asp Val Arg Glu Ala 100 105 110

Asp Ile Pro Phe Ala Tyr Arg Tyr Leu Ile Asp Lys Asp Leu Ala Cys 115 120 125

Met Asp Gly 130

<210> 27

<211> 135

<212> PRT

<213> Cenarchaeum symbiosum

<400> 27

Thr Val Gln Asp Ala Val Glu Ile Pro Pro Ser Leu Leu Val Ser Ala 1 5 10 15

Thr Tyr Asp Ser Gln Ala Gly Ala Val Val Leu Lys Phe Tyr Glu Pro 20 25 30

Glu Ser Gln Lys Ile Val His Trp Thr Asp Asn Thr Gly His Lys Pro 35 40 45

Tyr Cys Tyr Thr Arg Gln Pro Pro Ser Glu Leu Gly Glu Leu Glu Gly 50 60

Arg Glu Asp Val Leu Gly Thr Glu Gln Val Met Arg His Asp Leu Ile 65 70 75 80

Ala Asp Lys Asp Val Pro Val Thr Lys Ile Thr Val Ala Asp Pro Leu 85 90 95 Page 42

Ala Ile Gly Gly Thr Asn Ser Glu Lys Ser Ile Arg Asn Ile Met Asp 100 105 110

Thr Trp Glu Ser Asp Ile Lys Tyr Tyr Glu Asn Tyr Leu Tyr Asp Lys 115 120 125

Ser Leu Val Val Gly Arg Tyr 130 135

<210> 28

<211> 133

<212> PRT

<213> Sulfolobus acidocaldarius

<400> 28

Trp Ile Lys Glu Ala Glu Asp Gly Lys Val Tyr Phe Leu Leu Gln Val 1 5 10 15

Asp Tyr Asp Gly Lys Lys Ser Arg Ala Val Cys Lys Leu Tyr Asp Lys 20 25 30

Glu Gly Lys Lys Ile Tyr Ile Met Gln Asp Glu Ser Gly His Lys Pro 35 40 45

Tyr Phe Leu Thr Asp Ile Asp Pro Asp Lys Val Asn Lys Ile Thr Lys 50 55 60

Val Val Arg Asp Pro Ser Phe Asp His Leu Glu Leu Ile Asn Lys Val 65 70 75 80

Asp Pro Tyr Thr Gly Lys Lys Ile Arg Leu Thr Lys Ile Val Val Lys 85 90 95

Asp Pro Leu Ala Val Arg Arg Met Arg Ser Ser Leu Pro Lys Ala Tyr 100 105 110

Glu Ala His Ile Lys Tyr Tyr Asn Asn Tyr Val Tyr Asp Asn Gly Leu 115 120 125

Ile Pro Gly Leu Ile 130

<210> 29

<211> 133

<212> PRT

<213> Sulfurisphaera ohwakuensis

<400> 29

Trp Ile Lys Glu Ala Glu Glu Gly Lys Ser Tyr Phe Leu Leu Gln Val 1 5 10 15

Asp Tyr Asp Gly Lys Lys Ser Lys Ala Ile Cys Lys Leu Tyr Asp Lys 20 25 30

Glu Thr Lys Lys Ile Tyr Ile Leu Tyr Asp Asn Thr Gly His Lys Pro 35 40 45

Tyr Phe Leu Thr Asp Ile Asp Pro Glu Lys Val Asn Lys Ile Pro Lys 50 55 60

Val Val Arg Asp Pro Ser Phe Asp His Leu Glu Thr Val Ile Lys Ile 65 70 75 80

Asp Pro Tyr Ser Gly Asn Lys Ile Lys Leu Thr Lys Ile Val Val Lys 85 90 95

Asp Pro Leu Ala Val Arg Arg Met Arg Asn Ser Val Pro Lys Ala Tyr 100 105 110

Glu Ala His Ile Lys Tyr Phe Asn Asn Tyr Ile Tyr Asp Leu Gly Leu 115 120 125

Ile Pro Gly Leu Pro 130

<210> 30

<211> 132

<212> PRT

<213> Sulfolobus solfataricus

<400> 30

Trp Leu Glu Glu Ala Gln Glu Asn Lys Ile Tyr Phe Leu Leu Gln Val 10 10 15

20510452_1(8.21.08).TXT
Asp Tyr Asp Gly Lys Lys Gly Lys Ala Val Cys Lys Leu Phe Asp Lys
20 25 30

Glu Thr Gln Lys Ile Tyr Ala Leu Tyr Asp Asn Thr Gly His Lys Pro $35 \hspace{1cm} 40 \hspace{1cm} 45$

Tyr Phe Leu Val Asp Leu Glu Pro Asp Lys Val Gly Lys Ile Pro Lys 50 60

Ile Arg Asp Pro Ser Phe Asp His Ile Glu Thr Val Ser Lys Ile Asp 65 70 75 80

Pro Tyr Thr Trp Asn Lys Phe Lys Leu Thr Lys Ile Val Val Arg Asp 85 90 95

Pro Leu Ala Val Arg Arg Leu Arg Asn Asp Val Pro Lys Ala Tyr Glu 100 105 110

Ala His Ile Lys Tyr Phe Asn Asn Tyr Met Tyr Asp Ile Gly Leu Ile 115 120 125

Pro Gly Met Pro 130

<210> 31

<211> 133

<212> PRT

<213> Pyrodictium occultum

<400> 31

Lys Pro Leu Glu Ala Arg Asp Gly Val Glu Gly Phe Leu Leu Gln Thr 1 5 10 15

Met Tyr Asp Gly Glu Arg Gly Val Ala Ala Ala Lys Ile Tyr Asp Asp . 20 25 30

Arg Asn Gly Ile Val Tyr Val Tyr Phe Asp Arg Thr Gly Tyr Met Pro
35 40 45

Tyr Phe Leu Thr Asp Ile Pro Pro Asp Lys Leu Gln Glu Leu His Glu 50 55 60

Val Val Arg His Lys Gly Phe Asp His Val Glu Val Glu Lys Phe 65 70 75 80

Asp Leu Leu Arg Trp Gln Arg Arg Lys Val Thr Lys Ile Val Val Lys 85 90 95

Thr Pro Asp Val Val Arg Val Leu Arg Asp Lys Val Pro Arg Ala Trp 100 105 . 110

Glu Ala Asn Ile Lys Phe His His Asn Tyr Ile Tyr Asp Tyr Gly Leu 115 120 125

Val Pro Gly Met Lys 130

<210> 32

<211> 138

<212> PRT

<213> Aeropyrum pernix

<400> 32

Val Arg Glu Pro Trp Val Glu Ser Val Arg Gly Tyr Leu Leu Asp Val
1 10 15

Arg Tyr Asp Gly Ser Leu Gly Lys Ala Val Leu Met Leu Tyr Asp Pro
20 25 30

Ser Ser Gly Ser Leu Val Lys Trp Ala Asp Arg Thr Gly His Lys Pro 35 40 45

Tyr Phe Leu Thr Asp Ala Arg Pro Glu Asp Leu Arg Ala Ala Gly Val 50 55 60

Asp Val Ser His Asp Glu Ser Phe Leu Gln Tyr Asp Leu Val Glu Lys 70 75 80

Phe His Pro Ile Asp Arg Lys Leu Val Lys Leu Tyr Lys Ile Val Val 85 90 95

Ser Asp Pro Leu Ala Val Arg Arg Leu Arg Glu Lys Val Ser Ser Ala 100 105 110

Gly Phe Ser Val Trp Glu Ala Asp Ile Lys Tyr His His Asn Tyr Ile 115 120 125

Phe Asp Arg Gln Leu Ile Pro Gly Ile Leu

Page 46

<210> 33

<211> 14

<212> PRT

<213> Unknown

<220>

<223> amino acid motif where X can be any amino acid

<400> 33

Glu Xaa Xaa Ile Xaa Phe Tyr Xaa Xaa Xaa Tyr Xaa Xaa Asp $1 \hspace{1.5cm} 5 \hspace{1.5cm} 10$